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# Virus Research

journal homepage: www.elsevier.com/locate/virusres

# COVID-19: CADD to the rescue

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ARTICLE INFO

Keywords: COVID-19 Coronavirus CADD Zoonotic diseases SARS-CoV-2 Virtual screening

# ABSTRACT

The recent outbreak of the deadly COVID-19 disease, being caused by the novel coronavirus (SARS-CoV-2), has put the world on red alert as it keeps spreading and recording more fatalities. Research efforts are being carried out to curtail the disease from spreading as it has been declared as of global health emergency. Hence, there is an exigent need to identify and design drugs that are capable of curing the infection and hinder its continual spread across the globe. Herein, a computer-aided drug design tool known as the virtual screening method was used to screen a database of 44 million compounds to find compounds that have the potential to inhibit the surface glycoprotein responsible for virus entry and binding. The consensus scoring approach selected three compounds with promising physicochemical properties and favorable molecular interactions with the target protein. These selected compounds can undergo lead optimization to be further developed as drugs that can be used in treating the COVID-19 disease.

## 1. Introduction

The reemergence of the coronavirus has taken the world by storm such that on the 30th of January 2020, the World Health Organization (WHO) declared it a public health emergency and later in February 2020, WHO officially named the novel coronavirus disease as COVID-19 (World Health Organization, 2020). The SARS-CoV-2 is the fourth zoonotic coronavirus to emerge in the last twenty years. The first two, the Severe Acute Respiratory Syndrome (SARS-CoV) and the Middle East Respiratory Syndrome (MERS-CoV), appeared in 2002 (Zhong et al., 2003) and 2012 (Sousou, 2015) respectively while in 2017, the Swine Acute Diarrhea Syndrome (SADS-CoV) affected the swine livestock (Cui et al., 2019). These diseases are known to be zoonotic and being transmitted by bats (Drexler et al., 2014) and it is suggested that the novel coronavirus, that was first identified in Wuhan, China, (SARS-CoV-2) is not an exception as some researchers had earlier predicted that there might be another zoonotic coronavirus outbreak in early 2019 (Fan et al., 2019).

Like other similar virus, recent updates show that the SARS-CoV-2 now spread from man to man, although it is presumed to be zoonotic in origin. While the genetic research confirmed SARS-CoV-2 is originated in bats, there are other speculations that other wild animals could serve as intermediary between bats and man, with pangolins leading as primary suspects as the intermediary in the case of Wuhan coronavirus (Liu et al., 2019). SARS-CoV-2 spread like other cold viruses and early symptoms include, but not limited to, runny nose, severe cough, sore throat, difficulty in breathing, etc. With several precautions and awareness on the deadly virus and its spread, more cases of infection are being anticipated globally. The known cases are being managed with supplemental oxygen and conservative fluid administration as there is currently no approved vaccine or antiviral agent to treat the infection by SARS-CoV-2 and the concerned researchers are continuously working on developing a vaccine or drug for novel coronavirus (Li et al., 2020; Liu et al., 2019).

With the continuous rise in number of confirmed cases since the outbreak of the SARS-CoV-2, a fast and reliable tool such as computeraided drug design (CADD) is of the essence. CADD is a renowned tool in the pharmaceutical industry and it does not only save time but also helps to cut costs of designing drugs. Virtual screening (VS) is one of the methods used in CADD and it enables screening of many compounds in a relatively short time compared to the high throughput screening via laboratory experiments (Kapetanovic, 2008; Leelananda and Lindert, 2016; Macalino et al., 2015; Manas and Green, 2017; Melo-Filho et al., 2019). Moreover, molecular docking as well as machine learning, can be used in virtual screening and these further enable effectiveness of VS

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https://doi.org/10.1016/j.virusres.2020.198022

Received 12 February 2020; Received in revised form 30 April 2020; Accepted 11 May 2020 Available online 15 May 2020

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(Mori et al., 2012; Pereira et al., 2020). For example, VS was employed in the development of approved drugs such as Aggrastat, a fibrinogen receptor, and Cevoglitazar, an effective PPAR- $\alpha/\gamma$  dual agonist for diabetes treatment (Clark, 2008). Recently, the use of consensus scoring has been acknowledged to also improve the enrichment of true positives and improve hit rates (Charifson et al., 1999; Clark et al., 2002; Feher, 2006). Herein, we employed computer-aided drug design that entails the use of consensus scoring to combine both molecular docking and machine learning VS method to discover potential inhibitors of the surface glycoprotein of the SARS-CoV-2 which is responsible for virus binding and entry. Our results identify three compounds with promising physicochemical properties and favorable molecular interactions with the target protein, and by extension, these identified compounds can undergo lead optimization to be further developed as drugs that can be used in treating the COVID-19.

# 2. Methodology

#### 2.1. Target protein preparation

Due to the recent emergent of the coronavirus disease, there are not many crystal structures of high resolution of the virus. As at the time the bulk of this work was completed, there was no crystal structure of the spike protein. Hence, homology modeling (Haddad et al., 2020; Krieger et al., 2003; Xiang, 2006) was employed. However, as at the end of March 2020, there are currently about 100 crystal structures of the SARS-CoV-2 deposited in the protein databank (www.rscb.org, Burley et al., 2018). It is intended that the best among these structures, particularly the spike proteins (PDB ID: 6VSB, 6VYB, 6LZG, 6MOJ and 6VXX) (Lan et al., 2020; Walls et al., 2020; Wrapp et al., 2020), would be used for future works. The surface spike glycoprotein functions as the cell-attachment recognition site hence, the reason for its consideration as the target protein as this is the critical part of the virus responsible for virus entry and binding (Gralinski and Menachery, 2020). The target protein was prepared using homology modeling with the aid of the Raptor program (Peng and Xu, 2011). The template protein used for the homology model is PDB ID:  $5 \times 58$  (Yuan et al., 2017). The sequence of the glycoprotein utilized to build the homology model was retrieved from the National Center for Biotechnology Information (NCBI) GenBank database (Wu et al., 2020) with the accession number: MN908947. The modeled structure was validated using the Ramachandran plot analysis with the aid of RAMPAGE webtool (Lovell et al., 2003; Ramachandran and Sasisekharan, 1968) and PrankWeb server (Jendele et al., 2019) to validate the amino residued involved in the protein-ligand interactions. The ConSurf web program (Ashkenazy et al., 2016) was used for the multiple sequence alignment (MSA) analysis, conserved score and phylogentic analysis.

# 2.2. Virtual screening

The MCULE full database (Kiss et al., 2012) with exactly 44,704,142 compounds, as at that the time of this work, was used for the first virtual screening experiment. A blind docking (Grosdidier et al., 2009) was carried out, which covered the whole of the protein since no binding pocket has been determined from experiments yet with the following parameters -1.298, -7.617 and 191.965 for X, Y and Z axes respectively. These coordinates represent the binding site area. The MCULE database was filtered using drug-like properties as used in our earlier works (Onawole et al., 2018, 2017; Sulaiman et al., 2019) which include having a maximum of 5 halogen atoms, five chiral centers and ten rotatable bonds; a minimum of 10 heavy (non-hydrogen) atoms and a minimum of 1 aromatic ring; and lastly should not violate not more than one of the Lipinski's rule of five (RO5) (Lipinski, 2004; Lipinski et al., 1997). After the filtration, 100,000 compounds were screened randomly using Autodock VINA as the molecular docking tool (Trott and Olson, 2010). The diversity selection of these 100,000 compounds ensured that the maximum similarity (S) threshold was set to 0.85. This assured that none of the resulted molecules was more similar than S, based on the Tanimoto coefficient of similarity (Bajusz et al., 2015; Cerqueira et al., 2015). The top-scored compounds were kept and used



Fig. 1. Flowchart depicting the methodology.

for a second virtual screening with BindScope (Jiménez Luna et al., 2018; Mysinger et al., 2012; Skalic et al., 2018). The second virtual screening, BindScope, employed a machine learning technique (convoluted neural network). Recently, machine learning technique is gaining considerable attention in the drug discovery process as it is much faster than molecular docking. Moreover, having a different method rather than another molecular docking program with a different scoring method helps to reduce the number of false positives (Chen et al., 2018; Stevens, 2014). The latest iteration of DUD-E database (Mysinger et al., 2012) was used in the training of BindScope. This database was comprised of 22,886 active compounds and 50 similar decoys for each active and docked against 102 different targets (Koes et al., 2013). To ensure a fair benchmarking, the targets were clustered by employing a 70 % sequence similarity cut-off which was provided by blastclust in the RSCB PDB database (www.rscb.org, Burley et al.,

2018). To use BindScope, the target protein, the homology modeled structure of the spike protein of SARS-CoV-2 in this case, was uploaded in PDB to the web application alongside a set of docked ligands (in this case the top 500 scored ligands from the first virtual screening) in structure-data file (SDF) format. The results from the virtual screenings were combined for consensus scoring using the rank voting method (Feher, 2006) to select the compounds which appeared as top-scored in both virtual screenings. The consensus scoring method is known to improve HIT rates by reducing the chances of false positives (Charifson et al., 1999; Huang et al., 2010; Stevens, 2014; Yang and Hsu, 2005) and has been applied in finding potential inhibitors of protein kinase B in anti-cancer drug discovery (Forino et al., 2005). The Discovery studio program (BIOVIA, 2015) was used to visualize the molecular interactions of the selected HITS with that target protein. Fig. 1 shows the flowchart that depicts the entire process of selecting hit compounds.

2019_nCoV 1		MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFL	56
SARS 1		ILF.T.T.GSDLDRC.TFDDVQA.NQHT.SMEIDT.YL	60
2019_nCoV 57	7	PFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQS	116
SARS 63		YGT.NHTGI.K.IAVVVS.MNN.S	113
2019_nCoV 12	17	LLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFL	176
SARS 12	14	VI.ISRA.N.EL.DNFASKPMGTQTHTMIFDN.FI.DA.S	169
2019_nCoV 1	77	MDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINIT	236
SARS 1	70	L.VSE.SHKFLYV.KGYQDVSNT.K.IFKL	229
2019_nCoV 2	37	RFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPL	296
SARS 2	30	N.RAI.TAFS.AQDIGTSFK.TMDSQN	283
2019_nCoV 29	97	SETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRK	356
SARS 28	84	A.LSVEIDV.SGDVE	343
2019_nCoV 39	57	RISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTG	416
SARS 34	44	K	403
2019_nCoV 42	17	KIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAG	476
SARS 40	04	VMLTR.I.ATSTK.YL.HGK.RNVPFSPD	463
2019_nCoV 47	77	STPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKN	536
SARS 46	64	GK.TP-PALWNDYT.T.INN.	522
2019_nCoV 5	37	KCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVS	596
SARS 52	23	QP.S.R.QVS.F.SK.SS.A	582
2019_nCoV 55	97	VITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHV	656
SARS 55	83		642
2019_nCoV 65	57	NNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPT	716
SARS 64	43	DTDS.IT.	698
2019_nCoV 7:	17	NFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDK	776
SARS 69	99	SIVMANAAA	758
2019_nCoV 77	77	NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQ	836
SARS 75	59		818
2019_nCoV 83	9 Y	GDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQI	896
SARS 81		.ENDAAVSA.A	878
2019_nCoV 89 SARS 87	97 P	FAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNA	956 938
2019_nCoV 95 SARS 93	7 Q	ALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAA	1016 998
2019_nCoV 10	017 E	IRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFT	1076
SARS 99	09 .		1058
2019_nCoV 10	)77 T	APAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNT	1136
SARS 10	)59 .		1118
2019_nCoV 11	.37 V	YDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNES	1196
SARS 11	.19 .		1178
2019_nCoV 119	.97 L	IDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKF	1256
SARS 11	.79 .		1238
2019_nCoV 12 SARS 12	57 D	EDDSEPVLKGVKLHYT 1273 	

Fig. 2. Sequence alignment of the surface glycoprotein for both SARS-CoV-2 and SARS-CoV corona viruses. Identical residues are denoted by an "." beneath the consensus position.

#### 3. Results and discussion

## 3.1. Sequence alignment analysis

From the whole genome of the SARS-CoV-2, only the genomic sequence of the surface glycoprotein was considered as this is the critical part of the virus responsible for virus entry and binding (Gralinski and Menachery, 2020), followed by a multiple sequence analysis using the Needleman-Wunch pairwise alignment method (Altschul et al., 2005, 1997) to compare the surface glycoprotein of both the SARS-CoV-2 and the SARS-CoV. The surface glycoprotein for SARS-CoV-2 is made up of 1273 amino acids while the SARS-CoV is 1255. The SARS-CoV-2 is 76 % identical to the SARS-CoV (Fig. 2). That is, 970 amino acids present in SAR-CoV-2 are equally present in SARS-CoV. The dots depicted in the multiple sequence alignment (MSA) for SARS-CoV denote identical sequences with 2019-nCoV (i.e. SARS-CoV-2) while the differences are denoted in red (Fig. 2). The percentage similarity suggests how closely related these two viruses are and also gives insights into how successful approach used in curtailing the SARS-CoV may also be effective for the novel SARS-CoV-2.

Besides, further MSA was done to compare the spike protein of SARS-CoV-2 with other related surface glycoproteins (SARS-CoV) with the aid of the ConSurf server (Ashkenazy et al., 2016). The phylogenetic tree which shows the evolutionary connection between the SARS-CoV-2 and other related species (Fig. 3) denote that the spike protein of SARS-CoV-2 is closely related to other coronaviruses such as bat SARS and middle east respiratory syndrome (MERS) corona virus (Table S1) but it is closest to UniRef90 A0A2R3SUW7 (The UniProt Consortium, 2019) which is a Bat SARS-like coronavirus. The phylogentic tree suggests that SARS-CoV-2 may have originated from bats (Fan et al., 2019). The MSA of SARS-CoV-2 was done with the other 29 species depicted in the phylogentic tree (Table S8). The conservation score which is a score allotted to each amino acid in a MSA is used to determine how conserved the amino acid is. A score value of 9 (maroon color) means the amino acid is well conserved while a value of 1 (cyan color) means it is variable (Fig. 4). The conservation scores of SARS-CoV-2 (Table S2) denote that about 60 % of the amino acids are conserved, that is, they have a conservation score of at least 6.

# 3.2. Protein structure and validation

The tertiary structure of the surface glycoprotein shows a few  $\alpha$ -helices, many  $\beta$ -pleated sheets, and long random coils (Fig. 5a). The

Ramachandran plot analysis, which depicts the favored, allowed and outlier values of  $\psi$  against  $\phi$  angles for a particular amino acid (Ramachandran and Sasisekharan, 1968), was used to validate the structure of the protein. A good quality protein is expected to have an outlier of less than 5 % (Kleywegt and Jones, 1996). For the SARS-CoV-2 surface glycoprotein, the homology modeled structure showed 93.6 %, 5.5 % and 0.9 % in the favored, allowed and outlier regions respectively. The outlier region which is less than 5% validates the choice of protein structure for virtual screening analysis. The deeper and lighter shade of blue and orange depicts the favored regions and allowed regions respectively (Fig. 5b). The triangles and squares are the general/Pre-Pro/Proline amino acids whereas the crossed-x denotes the glycine amino acids. The eleven amino acids which make up the outliers are in red squares. They are all found in the General and Pre-Pro areas, and none occurred in the Glycine area. Nevertheless, the protein structure is good enough for further analysis.

# 3.3. Consensus scoring

The first virtual screening employed a molecular docking technique with the aid of VINA (Trott and Olson, 2010) and the top 500 scored compounds were used for a second virtual screening but this time, using a machine learning technique (CNN) in the BindScope web tool (Jiménez Luna et al., 2018). Only the top 500 compounds were considered as the rest of the 100, 000 compounds considered had negative binding scores with the target protein. This is evident in the docking scores of the last 100 compounds from the first virtual screening (Table S2). The top 25 scored ligands from the first virtual screening using VINA (Fig. 6) and the second virtual screening using BindScope (Fig. 7) were considered. This approach is known as the vote rank method in consensus scoring (Feher, 2006). The top scores in VINA correlate to the ligands with the highest binding energies, while the more negative values imply a stronger binding affinity to the target protein. Whereas, in BindScope, the top-scored ligands are based on probability where values close to 1 imply strong binding affinity and those close to 0 imply low binding affinity with the target protein. Three compounds namely MCULE-2442351665-0-1, MCULE-6855995445-0-2 and MCULE-4671321297-0-1 appear in the top 25 scored ligands for both VINA and BINDSCOPE. MCULE-2442351665-0-1 is the 15th and 13th top scored ligand in VINA and BINDSCOPE respectively while MCULE-6855995445-0-2 appears as the 24th and 17th top scored ligand in VINA and BINDSCOPE respectively. MCULE-4671321297-0-1 comes in as the 25th and 9th top scored ligand in VINA and BINDSCOPE



Fig. 3. The phylogenetic tree for SARS-CoV-2 (input protein sequence).

1	11	21	31	41	351	361	371	381	391
M VFLVLLPL	SSQCVNLTT	<b>RTQLPPA</b> TN	S TRG YYPD	KVERS VLHS	YAWNRK ISN	<b>CVADYSVLYN</b>	SASFSTFKCY	<b>GVSPTKLNDL</b>	<b>CFTNVYADSF</b>
ebbbbbbbbe	beeebeebee	eeeeebeeee	eeeebbbbee	ebbebebebb	bbbeeeebee	bebebbebbe	bbebbebebe	eeeeebbeb	bbbebbbebb
			f ss f	fs	f	s f	S	f	s f
51	61	71	81	91	401	411	421	431	441
T DIGLP PS	VTWFHAIHV	SGTNGTKRF	N V PRNDGV	F E SNI	IRGDEVRQ	A QTGKIAD	YNYKLP DFT	GOVIA NSNN	LDSKVG YN
beebbbbbeb	ebbebbbbee	eeeeeeebe	eeebebeebb	bbbbeeeeb	bbebeebeeb	eeeeeebbe	beeeeeeee	bbebbbeeee	eeeeeeeee
s			s s				ff f f	S	
101	111	121	131	141	451	461	471	481	491
TREWINGTT	DSCTOS TTV	NN T WYTKY	CEFORCNDEE	LGYYHKNNK	YLYBLERKSN	TREFERDUS	ETYOAGSTPC	NGEGENCYE	P OS GOPT
hebbbbbbbb	eeebebbbbbb	hebbbbbbb	bebebbeeeb	hebbeeebe	bebebeeeee	beeeeeeee	eebeeeeeb	eebeeebee	0000000000
5 55		f	sssf	2022000000	2020200000	f			
151	161	171	101	191	501	511	521	521	541
SMARSHOPVY	SS NNOTERY		CKOCN KN P	FURKNIDAY	NOVOYO NOV	MUT SEFT T UN	DATIO	TURNKOVNI	DNENCT TOTO
chhosehebb	sohoobbbboo	beechebeece	Chigon ha	shippensh	Roverg IRv	bbbbbbbbb	PAIVEGERKS	TALVANCOVIN	hebebbebeb
eppeeebepp	fe	peeepepeee	f	ebbbeeeeeb	eebeeebbeb	DDDDDDDDDDe	fee	f	
0.01	011	001	-	<b>5</b>		5.61	LSS	L 5	5 15 515
	ZII		Z31		551	561	571	581	591
K KHIPI	NLVRDLPQGE	ALEFLVD	I INTIKEQT	LLAINESTLI	VLTD SNKKFL	ProQUGRD A	DTTDAVRDEQ	TLEILDITEC	SEGGVSVITP
DDDDDDeDeeD	eppeebeebb	ebbeebbebb	Dependenen	DDDDeeeeee	ebeebeeebe	ebeebeeeee	eebeebeeee	ebebbbbeeb	bebebbbbbbe
		I S		I	Í	t s	1 11	İs	s
251	261	271	281	291	601	611	621	631	641
GDSSSGWTA	GAAAY VGYI	QPRTFLLKYN	NGTITDAD	CALDPISOTK	GTNT SNOVAV	LYQDVNCTEV	PVAIHADQLT	PTWRVYSTGS	NV QTRAGCL
eeeeeebee	ebbbbbbbeb	eebebbbebe	eeeebeebbe	beeeeeebe	beeeeebbb	bbeebebeee	eebbeeeee	eebebeeeee	eebebebbbb
	S	Ŧ	Í	s Í	sf s	s			fs ss
301	311	321	331	341	651	661	671	681	691
CTL SFTVEK	GIYQTSNFRV	QPTESI R P	NI NLOPFGE	VONATRFA V	GAEHVNNSY	<b>ECDIPIGAGI</b>	CASYQTQTNS	RRARSVAS	SIIAYTMSLG
bebeebebee	ebbebbebeb	eeeebbebe	ebeeebebee	bbeeeebeeb	bbbeeeebbe	ebebebbebb	bbeeeeeee	beeeeebee	bbbbbebeee
S	fssf sf f	f	S	f		S S	S		
701	711	721	731	741	1051	1061	1071	1081	1091
701 A NSVAYSNN	711 SIAIPTNETI	721 SVTTEILPVS	731 MTKTSVDCTM	741 YICGDSTECS	1051 SFPOSAPHGV	1061 VELHVTYVPA	1071 OEKNFTTAPA	1081 ICHDCKAHFE	1091 EGVEVSNGT
701 A NSVAYSNN eeeebbbbeb	711 SIAIPTNETI bbebbeebbb	721 SVTTEILPVS bbbeebbebb	731 MTKTSVDCTM beebebebee	741 YICGDSTECS ebbeccebe	1051 SFPQSAPHGV bbbeebeebb	1061 VELHVTYVPA bbbbbbbeee	1071 QEKNFTTAPA eccebebbeb	1081 ICHDCKAHFE bbeeeeebe	1091 ECVEVSNGT eebbbbbbee
701 A NSVAYSNN eeeebbbbeb	711 SIAIPTNETI bbebbeebbb s fs	721 SVTTEILPVS bbbeebbebb f f	731 MTKTSVDCTM beebebebee f fs f	741 YICGDSTECS ebbeeeebe f sfff s	1051 SFPQSAPHGV bbbeebeebb s f sffs	1061 VELHVTYVPA bbbbbbbeee s ss s ff	1071 QEKNFTTAPA eeeebebbeb fs	1081 ICHDCKAHFE bbeeeeebe ss f f	1091 ECVEVSNGI eebbbbbbbee fs s
701 A NSVAYSNN eeeebbbbeb	711 SIAIPTNETI bbebbeebbb s fs 761	721 SVTTEILPVS bbbeebbebb f f 771	731 MTKTSVDCTM beebebebee f fs f 781	741 YICGDSTECS ebbeceebe f sfff s 791	1051 SFPQSAPHCV bbbeebeebb s f sffs 1101	1061 VELHVTYVPA bbbbbbbbeee s ss s ff 1111	1071 QEKNFTTAPA eeeebebbeb fs 1121	1081 ICHDCKAHFE bbeeeeebe ss f f 1131	1091 ECVEVSNGI eebbbbbbbee fs s 1141
701 A NSVAYSNN eeeebbbbeb 751	711 SIAIPTNETI bbebbeebbb s fs 761 TOLNBALIGT	721 SVTTEILPVS bbbeebbebb f f 771 AVEOUKNTOE	731 MTKTSVDCTM beebebee f fs f 781 VFAOVKOTYK	741 TCGDSTECS ebbeccebe f sfff s 791 TPLT DEG F	1051 SFPQSAEHCV bbbeebeebb s f sffs 1101	1061 VELHVTYVEA bbbbbbbbeee s ss s ff 1111 FPO TT DYT	1071 QEKNFTTAPA eeeebebbeb fs 1121 F. SCNCDWIT	1081 ICHDCKAHFE bbeeeeebe ss f f 1131 CIVANTVYDE	1091 ECVEVSNGT eebbbbbbee fs s 1141
701 A NSVAYSNN eeeebbbbeb 751 NLL QYGSEC	711 SIAIPTNETI bbebbeebbb s fs 761 TQLNRALIGI	721 SVTTEILPVS bbbeebbebb f f 771 AVEQLKNTQE	731 MTKTSVDCTM beebebebee f fs f 781 VFAQVKQUYK	741 <b>MICGDSTECS</b> ebbeceebe f sfff s 791 <b>TPEI DFG F</b> ecobeceeb	1051 SFPQSAPHCV bbbeebeebb s f sffs 1101 HWFVTQRNFY	1061 VGL:VTYVPA bbbbbbbeee s ss s ff 1111 EPQ IT DNT	1071 OEKNFTTAPA eeeebebbeb fs 1121 F SGNCDVVI	1081 ICHDCKAHFE bbeeceebe ss f f 1131 GIVNNTVYDE	1091 ECVEVSNGT ebbbbbbbe fs s 1141 QPELDSEKE
701 A NSVAYSNN eeeebbbbeb 751 NLI OYESIC ebbeebbebb	711 SIAIPTNETI bbebbeebbb s fs 761 TQLNRALIGI eebeebbeeb f ss	721 SVTTEILPVS bbbeebbebb f f 771 AVEQIKITQE bbeeeebee ff f	731 MTKTSVDCTM beebebebee f fs f 781 VFAQVKQTYK bbebeeeee f	741 YICGDSTECS ebbeccebe f sfff s 791 TPEI DFG F eccbecceb	1051 SFPQSAPHCV bbbeebeebb s f sffs 1101 EWFVTQRNFY ebbbbeebe	1061 VELEVTYVEA bbbbbbbeee s ss s ff 1111 EPQ IT DNT eeebeeee	1071 OEKNFTTAPA eeeebebbeb fs 1121 F SGNCDVVI eebeebebeb	1081 ICHDCKAHFE bbeeceebe ss f f 1131 GIVNNTVYDE ebbeece	1091 ECVEVSNGT eebbbbbbee fs s 1141 QPELDSEKE eeeeeeebe
701 A NSVAYSNN eeeebbbbeb 751 NLP OYCSIC ebbeebbebb s fss ss	711 SIAIPTNETI bbebbeebbb s fs 761 TOINRAL GI eebeebbeeb f ss	721 <b>SVTTEILPVS</b> <b>bbbeebbebb</b> <b>f</b> 771 <b>AVEODKNTOE</b> <b>bbeeeebee</b> <b>ff</b> <b>f</b> 921	731 MTKTSVDCTM beebebebee f fs f 781 VFAQVKQTYK bbebeeee f	741 <b>MICODSTECS</b> ebbeeeebe f sfff s 791 TPEI DFG F eeebeeeeb	1051 SFPQSAPHCV bbbeebeebb s f sffs 1101 FWFVTQRNFY ebbbbeeebe sf	1061 VELEVTIVEA bbbbbbbbeee s ss s ff 1111 EFQ IT DNT eeebeeeee f f f	1071 DEKNFTTAPA eccebebeb fs 1121 F SGNCDVVI ecbecbebeb	1081 <b>ICHD</b> CKAHFE bbecceebe ss f f 1131 GIVNNTVYDE ebbecebeee f f	1091 ECVINSNGT eebbbbbbee fs s 1141 QPELDSikke eeeeeebee s
701 A NSVAYSNN eeebbbbbb 751 NLI OYCSIC ebbeebbebb s fss ss 801	711 SIAIPTNETI bbbbbeebbb s fs 761 TQINRAL GI eebeebbeeb f ss 811	721 SVTTOILEVS bbbebbbbb f f 771 AVEORNTOE bbeeeebee ff f 821 UEBEACATTER	731 MT&TSVDCTM beebebebee f fs f VFAQVKQTYK bbeebeeeee f 831 QANKQVCCP	741 MICODSTEGS cbbeecebe f sfff s 791 TPEI DFG F ccebececeb 841	1051 SFPQSAPHCV bbbeebeebb s f sffs 1101 FWFVTQRNFY cbbbbeeebe sf 1151	1061 VELHVTYVPA bbbbbbbeee s ss s ff 1111 EPQ IT DNT eeeebeeee f f f 1161	1071 OEKNFTTAPA cecebebeb fs 1121 F SGNCDVVI cebeebebeb 1171	1081 TehDeKAHFF bbeeeeebe ss f f 1131 GIVNNTVYDE ebbeeebeee f f 1181	1091 ECVEVENGT ebbbbbbee fs s 1141 QPELDS5KE eeeeeebee s 1191
701 A SVAYSNN eeeebbbbbb 751 NLP OXCSIC ebbebbebb s fss ss 801 NESQILPDPS	711 SIAIETNETI bebebebbe s fs 761 TOINRAL GI ebebbeeb f ss 811 KESKREIED	721 SVTTBILPVS bbbebbbb f f 771 AVEQDKNTQE bbeeeebee ff f 821 MFNKVTLAD	731 MTXTSVDCTM beebebebee f fs f 781 VFAQVKQTYK bbeebeeeee f 831 AGSUKQYGDC	741 <b>MICODSTEGS</b> ebbeecebe f sfff s 791 <b>TPET DFG F</b> ecebeecebe 841 <b>LGD TARED</b>	1051 SFPQSAPHCV bbbeebeebb s f sffs 1101 WFVTQRNFY ebbbbeeebe sf 1151 FLDKYFKNHT	1061 VELHVTVVPA bbbbbbbeee s ss s ff 1111 EPQ IT DAT eccebeeee f f f 1161 SPDVDL DIS	1071 OENFTAPA eccebebebe fs 1121 F SGNCDVVI ecbecbebeb 1171 INASVVNIQ	1081 TehDeKAHFE bbeeeeebe ss f f 1131 GIVNNTVYDE ebbeebeee f f 1181 KETERINEVA	1091 ECVENSING ebbbbbbee fs s 1141 QPELSSKE eeccebee s 1191 KNIASESLIDH
701 A NSVAYSNN eeeebbbbeb 751 NLT OXCSTC ebbeebbebe s fss ss 801 NESQILPDPS ebbebbeeee	711 SIAIETNETI bbebbeebbb s fs 761 TQINRAL GI eebeebbeeb f ss 811 KESKRSFIED eeeeebbee	721 SVTTDILPVS bbbeebebbb f f 771 AVEORNTQE bbeecebeb 821 MFNKVTLAD bbbeebebbb	731 MT&TSVDCTM beebebeee f fs f 781 VFAQVKQIYK beebeeeee f 831 AGSTKQIGDC eebbeeeee	741 <b>ICCDSIECS</b> ebbeccebe f sfff s 791 <b>TPLI DFG F</b> ecebecceb 841 <b>ICDIAARDII</b> ecebecceb	1051 SFPQSACHCV bbbeebeebb s f sffs 1101 WFVTQRNFY ebbbeeebe sf 1151 FIDKYFKNHH	1061 VELHVTYVPA bobbbbbeee s ss sf 1111 EFQ IT DNT eeeebeeee f f f 1161 SPDVDL DIS	1071 OEKNFTTAPA Seceebebbe fs 1121 F SGNCDVVI sebeebebeb 1171 INASVVNIQ sbeebbbbbebe	1081 <b>ICHD KAHFE</b> bbecceebe ss f f 1131 <b>GIVNNTVYD</b> ebbeccbece f f 1181 <b>KEIPRINEVA</b> eebecbecbb	1091 ECVENENGT ebbbend fs s 1141 QPEL_SEKE ecceeded s 1191 KNINESLIDI ecbeebben
701 A NSVAYSNN eeeebbbbbb 751 NLI OXCSFC ebbebbebb s fss ss 801 MESQILPDPS ebbebbeece fs f	711 SIALETNETI bbebbeebbe s fs 761 TQINRALIGI eebeebbeeb f ss 811 KESKRSFIED eeeeebbee ff ff	721 SVTTOILEVS bbbeebbebb f f 771 AVEOKNTQE bbeeebebb ss ff s 022	731 MTKTSVDCTM beebebebee f fs f 781 VFAQVKQIYK bbeebeeeee f 831 AGIIKQIGDC eebbeeeeb ffssf f s	741 <b>MCGDSAEGS</b> ebbeceebe f sfff s 791 <b>TPEI DFG F</b> eccebeceeb 841 <b>LGD AARDTI</b> eccebecebb ffs	1051 SFPOSACHCV bbbeebeebs s f sffs 1101 WFVTORNFY ebbbbeeebe sf 1151 FLDKYFKNHM ebeebbeeee f f fff	1061 V5LHVTVVPA bbbbbbbeeee s ss s ff 1111 EPQ IT DNT eeeebeeeee f f f 1161 SPDVDL DIS eeeebeebe f f	1071 OEKNFTTAPA seebebbeb 1121 F SGNCDVVI sebebbbbebb sf s	1081 TCHDCKAHFE bbeccebe ss f f 1131 GIVNNTVYDE ebbeccbece f f 1181 KEIDRINEVA ecbecbecbb f s ss	1091 ECVIVENCT ebbbbbbbe fs s 1141 QPEL_SSKE ecceeded s 1191 KNIVESLIDD ecbebbber sf s sfs
701 A NSVAYSNN eeeebbbbbeb 751 NII OYCSFC ebbeebbebb s fss ss 801 NISOILPDPS ebbebbeeee fs f 851	711 SIAIETNETI bbebbebbe s fs 761 TQLNRAL GI eebeebbeeb f ss 811 KESKRSFIED eeeeeebbee ff ff 861	721 SVTTOILEVS bbbeebbebb f f 771 AVEOKNTOE bbeeebebb ss ff s 871 ss ff s	731 MTXTSVDCTM beebebebee f fs f 781 VFAQVKQTYK bbeebeeeee f 831 AGDIKQXGDC eebbeeeeb ffssf f s 881	741 MICGDSAEGS cbbeeceebe f sfff s 791 TPEI DFG F ceeebeeceeb 841 LGDIAARDII ceeebeebb ffs 891	1051 SFPOSACHCV bbbeebeebb s f sffs 1101 EWFVFORNFY ebbbbeeebe sf 1151 EIDKYFKNHM ebeebbeeee f f fff 1201	1061 VSLHVTYVPA bbbbbbbeee s ss s ff 1111 EPQ IT DNT eccebecee f f f 1161 SPDVDL DIS eccebecbe f f 1211	1071 OEKNFTTAPA seeebebbeb fs 1121 F SGNCDVVI sebeebebbeb 1171 INASVVNIQ sbebbbbbeb sf s 1221	1081 TCHDCKAHFE bbececebe ss f f 1131 CIVNNTVYDE ebbecebece f f 1181 KCTRINEV2 ecbecebeb f s ss 1231	1091 ECVINIST ebbbbbbee fs s 1141 OPELISIKE eeceechee s 1191 KNINESLID eebebbee sf s sfs 1241
701 A NSVAYSNN eeeebbbbbb 751 NLI OYCSIC ebbebbebb s fss ss 801 NISOILPDPS ebbebbeeee fs f 851 CACKENCLIY	711 SIAIETNETI bebeebbeeb s fs 761 TQLNRAL GI eebeebbeeb f ss 811 KESKRSFIED eeeeebbee ff ff 861	721 SVTTOILPVS bbbeebbebb f f 771 AVEOKNTOE bbeeebebe ss ff s 871 AVENTIAD bbeeebebbb ss ff s 871 AOVISAILAG	731 MTXTSVDCTM beebebebee f fs f 781 VFAQVKQTYK bbeebeeeee f 831 AGDIKQIGDC eebbeeeeb ffssf f s 881 TITSG FFGA	741 MICODSIEGS cbbeececbe f sfff s 791 TPEI DFG F ccebeccecb 841 LGD AARDII ccecebecbb ffs 891 GALQIPEAN	1051 SFPQSAPHCV bbbeebeeb s f sffs 1101 EWFVVQRNFY ebbbbeebe sf 1151 ELDKYFKNHJ ebeebbeeee f f fff 1201 QETGKYEQYI	1061 VSLHVTVVPA bbbbbbbeee s ss s ff 1111 EPQ IT DNT eeeebeeee f f f 1161 SPDVDL DIS eeeebeebe f f 1211 KWEVYINTES	1071 OENFTTAPA eccebebbeb fs 1121 F SGNCDVVI ecbebbebeb 1171 NASVVNIQ ebebbbebe sf s 1221 IAGLIAIVMV	1081 TGHD KAHFF bbeccecbe ss f f 1131 GIVNNTVYDE ebbeccbece f f 1181 KEIRINEVA eebecebb f s ss 1231 TIMLCCMISC	1091 ECVENSING ebbbbbbee fs s 1141 QPELDSEKE eeeeeebee s 1191 KNINESLID eebeebbee sf s sfs 1241 CSC KCCCS
701 A NSVAYSNN eeeebbbbbbb 751 NLT OXCSTC ebbeebbebb s fss ss 801 NESQILPDPS ebbebbeeee fs f 851 CACKFNCLTY bbeebeebeb	711 SIAIETNETI bbebbeebbb s fs 761 TQLNRALIGI eebeebbeeb f ss 811 KESKRSFIED eeeeebbee ff ff 861 IPPLLTDEMI bbebbeebe	721 SVTTBILPVS bbbebbbbb f f 771 AVEOKNTOE bbeecebec ff f 821 MFNKVTLAD bbeebbbbb ss ff s 871 AQVTSAMAG bbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbb	731 MT&TSVDCTM beebebebe f fs f 781 VFAQVKQIYK beebeeeee f 831 AGSIKQIGD eebbeeeeb ffssf f s 881 TITSG FFGA bbebbbbbbb	741 <b>ICCDS:ECS</b> ebbeceebe f sfff s 791 <b>TPEI DFG F</b> eccebeeceb 841 <b>ICD:AARDII</b> eccebeebe ffs 891 <b>CFALQIEPAM</b> bbbbbbbbbbb	1051 SFPOSACHCV bbbeebebb s f sffs 1101 WFVTORNFY ebbbbeeebe sf 1151 ETDKYFKNHH ebeebbeeee f f ff f 1201 OETGKYEQYI cebeebeeeb	1061 VELHVTYVPA bobbbbbeee s ss s ff 1111 EFQ IT DNT eeeebeeee f f f 1161 SPDVDL DIS eeeebeebe f f 1211 KWEWYIWIGE ebbbbbbbbbb	1071 OEKNFTTAPA Seebebbb fs 1121 F SGNCDVVI sebebbbbbbb sf s 1221 INASVVNIQ sbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbb	1081 Teho KAHFE bbeeceebe ss f f 1131 GIVNNTVYDE ebbeecbeebe f f 1181 KEIRINEVA eebeebeebb f s ss 1231 TIMLCCMISC bbbbbbbeee	1091 ECVENENGT ebbbbbbe fs s 1141 QPEL_SEKE eeceecees s 1191 KNINESLIDI eebeebber sf s sfs 1241 CSC KCCCS
701 A NSVAYSNN eeeebbbbbbb 751 NLI OYCSFC ebbebbebb s fss 801 NESQILPDPS ebbebbeeee fs f 851 CACKFNCLTV bbeebeebb ssf ff fs	711 SIALETNETI bbebbeebbeeb f ss 811 KESKRSFIED eeeeebbee ff ff 861 iPPLLITDEXI bbebbeeebe ssf s	721 SVTT3ILPVS bbbebbbb f f 771 AVEOKNTQE bbeecebee ff f 821 FNKVTLA bbeecebebbb ss ff s 871 AQVTSALLAG bbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbb	731 MTKTSVDCTM beebebebe f fs f 781 VFAQVKQIYK beebeeeee f 831 AGIKQIGD ffssf f s 881 TITSG FFGA beebbbbbbb s	741 ICCDSACCS ebbeceebe f sfff s 791 TPEI DFG F ecebeceebe 841 ICDIAARDIT ecebecebe ffs 893 67ALQIPFAM bbbbbbbbbb s ssss	1051 SFD0SACHCV bbbeebeebs s f sffs 1101 WFVTORNFY ebbbbeeebe sf 1151 ELDKYFKNHH cbeebbeeee f f fff 1201 OBIGKNEONI ceebecbeeeb f sf s f	1061 V5LHVTYVPA bbbbbbbeee s ss s ff 1111 EPQ IT DNT eccebeeee f f f 1211 KWEVYLIGE ebbbbbbbb f sss ss s	1071 OENFTAPA ceebebbeb fs 1121 F SGNCDVVI cebebbbbebb sf s 1221 INASVVNIQ cbebbbbbbbb sf s 1221 IAGUIAIVMV bbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbb	1081 ICHD KAHFE bbeeceebe ss f f 1131 GIVNNTVYDE ebbeecbeebe f f 1181 KEIERINEVA eebeebeebb f s ss 1231 TIMLCCMISC bbbbbbbeee	1091 ECVEVENET ebbbbbbe fs s 1141 QPEL_SSKE eeceecee s 1191 KNINESLIDH eebeebber sf s sfs 1241 CC KCCCS eeebeeber ff f fs
701 A NSVAYSNN eeeebbbbbbb 751 NII OYCSFC ebbebbbbb s fss ss 801 NISQILPDPS cbbebbeeee fs f 851 CACKFNCLTV bbeebeebeb ssf ff fs 901	711 SIAIETNETI bbebbeebbeeb f ss 811 KESKRSFIED eeeeeebbee ff ff 861 i2ELITDE I bbebbeebe ssf s 911	721 SVTTOILPVS bbbeebebb f f 771 AVEOKNTQE bbeeeebee fff 821 infFNKVTLAD bbeeebebbb ss ff s 871 AQTISALTAG bbbbbbbbbbb ss s 921	731 MTGTSVDCTM beebebebee f fs f 781 VFAQVKQIYK bbeebeeeee f 831 AGDIKQYGD ffssf f s 881 TITSG IFGA bbebbbbbb s 931	741 MCGDSAEGS cbbeeeebe f sfff s 791 TPEI DFG F ceeebeeeeb 841 LCD AARDIT ceeebeebb ffs 891 CALQIEPAM bbbbbbbbbb s ss ss 941	1051 SFPOSACHCV bbbeebeeb s f sffs 1101 WFVJORNFY ebbebbeeebe sf 1151 SIDKYFKNHM ebbebbeeee f f fff 1201 OFFGKMEOWI cebeebeeeb f sf s f 1251	1061 V5LHVTYVPA bbbbbbbeee s ss s ff 1111 EPQ IT DNT eecebecee f f f 1161 SPDVDL DIS eecebecbe f f 1211 KWEWMINEG ebbbbbbbbb f ss ss s 1261	1071 OEKNFTTAPA ceeebebbeb fs 1121 F SGNCDVVI cebebbbbebe sf s 1221 FACTIAIVMV bbbbbbbbbbb sss 1271	1081 TCHDCKAHFE bbeeeeeee ss f f 1131 ebbeeebeee f f 1181 KEIDRINEVA eebeebeebb f s ss 1231 TIMLCCMISC bbbbbbeeee fff	1091 ECVIVISNOT ebbbbbbee fs s 1141 QPEL_SSKE eeceecebee s 1191 KNINESLID eebebbbee sf s sfs 1241 CSC KCCSC eebeebee ff f fs
701 A NSVAYSNN eeeebbbbbb 751 NLI OYCSIC ebbebbebb s fss ss 801 NESOILPDPS ebbebbeeee fs f 851 CACKFNCLTY bbebebbbs ssf ff fs 901 OMAYRFNCIG	711 SIAIETNETI bebebebeb s fs 761 TQLNRAM GI eebeebbeeb f ss 811 KESKRSFIED eeeeeebbee ff ff 861 IPPLITDEMI bebebeebe ssf s 911 VTONVLYSNO	721 SVTTOILPVS bbbeebebb f f 771 AVEOKNTOE bbeeeebee ff f 821 AFNKVTLAD bbeeebebbb ss ff s 871 AOVISAINAG bbbbbbbbbbbbbb ss s 921 KLIANGINSA	731 MTXTSVDCTM beebebebee f fs f 781 VFAQVKQTYK bbeebeeeee f 831 AGDIKQXGDC ffssf f s 881 TTTSG FFGA bbebbbbbbb s 931 I KTODSLSS	741 XICCDSAECS cbbeeceebe f sfff s 791 TPLI DFG F ceeebeeceebe 841 LGD AARDIT ceeeebeebb ffs 891 GALQITEPAM bbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbb	1051 SFPOSACHCV bbbeebeeb s f sffs 1101 EWFVVORNEY ebbbbeebe sf 1151 EIDKYFKNHi ebeebbeeee f f fff 1201 OBTCKYEQYI ebeebeeeb f sf s f 1251 GSGG FDEDD	1061 VSLHVTYVPA bbbbbbbeee s ss s ff 1111 EPQ IT DNT eecebeeee f f f 1161 SPDVDL DIS eecebeebe f f 1211 KWENYINTEP ebbbbbbbb f ss ss s 1261 SEPVLKGVKL	1071 OENTTAPA eeeebebebe fs 1121 F SGNCDVVI eebebebebe sf s 1221 IACLIAIVMV bbbbbbbbbb sss 1271 HYT	1081 TehDeKAHFF bbeeeeebe ss f f 1131 ebbeebeee f f 1181 KEIDRINEVA eebeebb f s ss 1231 TIMLCCMISC bbbbbbeeee fff	1091 ECVINIST ebbbbbbee fs s 1141 QPELSSKE eeeeebee sf s sfs 1241 SC KCCCS eeebeebeb ff f fs
701 A NSVAYSNN eeeebbbbbbb 751 NLT OXCSTC ebbebbebb s fss ss 801 NESOILPDPS ebbebbeeee fs f 851 CACKFNCLTY bbeebeebeb ssf ff fs 901 CMAYRFNCIG	711 SIAIETNETI bbebbeebb s fs 761 TQLNRALIGI eebeebbeeb ff ss 811 KESKRSFIED eeeeebbee ff ff 861 IPPLITDEVI bbebbeeebe ssf s 911 VTONVIYENC	721 SVTTEILPVS bbbeebebb f f 771 AVEORNTOE bbeeeebee ff f 821 MFNKVTLAD bbeebebbb ss ff s 871 AQVTSALAAG bbbbbbbbbbb ss s 921 KLIANGENSA eebbeebeeb	731 MTKTSVDCTM Debebebee f fs f 781 VFAQVKQIYK bbebeeeee f 831 AGSIKQIGD eebbeeeeb ffssf f s 881 TITSG FFGA bbebbbbbbb s 931 I KTODSLSS bebeeeeeb	741 ICCDSIECS ebbeccebe f sfff s 791 TPLI DFG F eccebecebe ffs 841 ICDIAARDII eccebecbe ffs 891 CALQUIPAM bbbbbbbbbb s ss ss 941 IASAIGKLOD ebebbecbee	1051 SFPOSACHCV bbbeebeebe s f sffs 1101 HNFVTORNFY ebbbbeebe sf 1151 ETDKYFKNHH ebeebbeeee f f ff ff 1201 OETGKYEQYI cebeebeeeb f sf s f 1251 GSGC FDEDD cebebeeeee	1061 VELHVTYVPA bobbbbbeee s ss sff 1111 EFQ IT DNT eccebeeeee f f f 1261 SPDVDL DIS eccebeebe f f 1211 KWEWYIWIGE ebbbbbbbbbb f sss ss s 1261 SEPVLKGVKI	1071 OEKNFTTAPA Seebebbeb fs 1121 F SGNCDVVI eebeebebeb 1171 INASVVNIQ ebebbbbbebe sf s 1221 IACLIAIVMV bbbbbbbbbbb sss 1271 HYT eeee	1081 <b>ICHD KAHFE</b> bbececebe ss f f 1131 <b>GIVNNTVYD</b> ebbeebeebe f f 1181 <b>KEIPRINEVA</b> eebeebeebb f s ss 1231 <b>TIMLCCMISC</b> bbbbbbbeee fff	1091 ECVENENGT ebbbbbe fs s 1141 QPEL_SEKE eeeeeebee s 1191 KNNESLIDI eebeebber sf s sfs 1241 CSC KCCCS eeebeeber ff f fs
701 A NSVAYSNN eeeebbbbeb 751 NLI QYCSTC ebbebbebb s fsss 801 NESQILPDPS ebbebbeeee fs f 851 CACKENCLIV bbeebebbb ssf ff fs 901	711 SIALETNETI bbebbeebb s fs 761 TQINRALIGI eebeebbeeb ff ss 811 KESKRSFIED eeeeebbee ff ff 861 iPPLLITDEMI bbebbeeebe ssf s 911 VTONVLYENC	721 SVTT3ILPVS bbbeebebb f f 771 AVEOKNTQE bbeeeebebb ss ff s 871 bbbebbbbbbbb ss s 921 KLIANOENSA gebbeebebb f ssffsf s	731 MTKTSVDCTM beebebebe f fs f 781 VFAQVKQIYK bbebbebee f 331 AGIKQIGD eebbeeeeb ffssf f s 881 TITSG FFGA bbebbbbbbbb s 931 I KTODSISS beebeeeeb f ff	741 CCCDSIECS ebbeeeeeb f sfff s 791 TPTIDFG F eeeebeeeb ffs 841 LCDIAARDIT eeeebeebb ffs 893 63ALQIPEAM bbbbbbbbbbb s ss ss 941 TASALGKLOD ebebbeebee f s f f	1051 SFPOSACHCV bbbeebeebs s f sffs 1101 WFVTORNFY ebbbbeeebe sf 1151 EDDKYFYKHT beebbeeee f f fff 1201 OBIGKXEOYI cebeebeeeb f sf s f 1251 GSCC FDEDD cebbeecees s ff	1061 V5LHVTYVPA bbbbbbbeee s ss sff 1111 EPQ IT DNT eeeebeeee f f f 1211 SPDVDL DIS eeeebeebe f f 1211 KWEVYINIGS f sss ss s 1261 SEPVLKGVKL beebbebbeb	1071 OEKNFTTAPA ceeebebbeb fs 1121 F SGNCDVVI eebeebebeb sf s 1221 IASVVNIQ ebebbbbebe sf s 1221 IAGIAIVMV bbbbbbbbbb sss 1271 HYT eee ff	1081 ICHD KAHFE bbeceebe ss f f 1131 GIVNNTVYD ebbeebee f f 1181 KSIERINEVA eebeebeebb f s ss 1231 TIMLCCMISC bbbbbbeee fff	1091 ECVEVENCT ebbbbbe fs s 1141 QPEL_SSKE eeeeeebe s 1191 KNINESLIDH eebeebbeh sf s sfs 1241 CSC KCCCS eeebeebeh ff f fs
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Fig. 4. The sequence the surface glycoprotein for SARS-CoV-2 showing the conservation score. The color scale represents the conservation scores where '9' implies highest conserved and '1' means highest variable.



Fig. 5. The tertiary structure (A) and Ramachandran plot (B) of the surface glycoprotein of SARS-CoV-2.



Fig. 6. The top 5% scored ligands from the first virtual screening (VINA).



Fig. 7. The top 5% scored ligands from the second virtual screening (BindScope).





Fig. 9. The oral bioavailability radar of the selected ligands (((A) compound A, (B) compound B, and (C) compound C). The colored zone is the suitable physicochemical space for oral bioavailability.

respectively. The selected ligands, MCULE-2442351665-0-1 (benzylfuran-2(5 H)-one), MCULE-6855995445-0-2 [((2,5-difluorophenyl) thio)-2,2-difluoroacetic acid)] and MCULE-4671321297-0-1 [(2-methylfuran-3-yl)methanesulfonyl fluoride] are henceforth referred to as compounds A, B and C in the subsequent sections of this article.

## 3.4. Physicochemical and ADMET assessment of selected ligands

Fig. 8 presents the chemical structure of the three selected compounds. While each of the three compounds has oxygen atoms, only compound A has two rings and the other two have one ring each. All the three compounds comply with the Lipinski's RO5 (Lipinski, 2016; Lipinski et al., 2001). The oral bioavailability radar (Fig. 9) shows that the colored zone is the perfect space for the physicochemical space. The LIPO (Lipophilicity) is derived from the XLOGP3 parameter (Table 1) and is expected to be in the range of -0.7 to +5.0. The values for all the

#### Table 1

Ligand	Compound A	Compound B	Compound C
Formula	$C_{11}H_{10}O_2$	$C_8H_4F_4O_2S$	C <sub>6</sub> H <sub>7</sub> FO <sub>3</sub> S
VINA	-4.9	-4.6	-4.6
BINDSCOPE	0.9919	0.9896	0.9929
Mass	174.2	240.17	178.18
#Heavy atoms	13	15	11
#Rotatable bonds	2	3	2
#H-bond acceptors	2	6	4
#H-bond donors	0	1	0
TPSA	26.3	62.6	55.66
XLOGP3	1.69	2.97	1.1
WLOGP	1.71	4.42	2.74
ESOL Log S	-2.19	-3.3	-1.84
ESOL Class	Soluble	Soluble	Very soluble
Lipinski #violations	0	0	0
Bioavailability Score	0.55	0.56	0.55
PAINS #alerts	0	0	0
Synthetic Accessibility	2.23	2.29	2.87

selected compounds fall within this required range and so are in the colored region. For the SIZE, it is expected not to exceed 500 gmol<sup>-1</sup> according to Lipinski's RO5, of which all the compounds obey. The POLAR (polarity) is determined by the Total Polarity Surface Area (TPSA) and the recommended range is between 20–130 Å<sup>2</sup>, within which all the selected ligands fall. The INSOLU (insolubility) category shows that all the selected ligands are soluble as they all fall between the range of 0 and 6 for their log S (ESOL) values. Ditto for the FLEX (flexibility) which is determined by the number of rotatable bonds and is expected not to exceed nine. However, the INSATU (Insaturation) requirement which is determined by the fraction of carbon sp<sup>3</sup> (Csp<sup>3</sup>) is expected to be in the range of 0.25 and 1, and this is met by compound C only. Hence, compound C has the best oral bioavailability since all its physicochemical parameters are in the colored zone.

Table 2 presents the results of the ADMET (absorption, distribution, metabolism, excretion, and Toxicity) analysis that was done using the AMDETSAR and SWISS ADME web tools (Cheng et al., 2012; Daina et al., 2017; Yang et al., 2018). The green-colored cells indicate excellent ADMET properties; the blue means good while the yellow and pink signify caution is needed and slightly dangerous respectively. These color codes may help during lead optimization to know what properties need to be modified. For all three selected ligands, they have good absorption properties, particularly concerning their human oral bioavailability which has been earlier suggested from the bioavailability radar. For distribution, the selected ligands are all permeants of the blood-brain barrier (BBB). Furthermore, the selected ligands are not substrate for the P-gp or the multidrug resistance protein that is responsible for transporting substances across the cell membrane. Hence, they can quickly move across the cell membrane. The metabolism of the selected compounds was predicted for cytochrome P450 inhibitors which catalyze many reactions involve in the metabolism of drugs. Compounds A and B are predicted to be inhibitors of CYP1A2 inhibitor while for the other cytochromes, all three selected ligands are non-inhibitors. The inhibition of Compounds A and B will increase plasma concentration and may lead to adverse outcomes. However, for all other cytochrome P450, the selected ligands are non-inhibitors which

## Table 2

The ADMET predictions of the selected ligands: compounds A, B, and C.

ADMET Absorption	Compound A	Compound B	Compound C
Absorption	Remark (Probability)	Remark (Probability)	Remark (Probability)
Human Intestinal Absorption (HIA)	Good (0.99)	Good (0.91)	Good (0.96)
Human oral bioavailability (HOB)	Good (0.76)	Safe (0.63)	Safe (0.79)
Caco-2 permeability	Good (0.91)	Good (0.60)	Good (0.65)
Distribution			
Plasma protein binding	Good (0.77)	Good (0.97)	Good (0.89)
BBB permeant	Yes	Yes	Yes
P-glycoprotein (P-gp) substrate	No	No	No
Metabolism			
Cytochrome (CYP450)			
CYP1A2 inhibitor	Yes	Yes	No
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	No	No	No
Excretion			
No info available	-	-	-
Toxicity			
Organ Toxicity			
Human ether-a-go-go inhibition	Safe (0.71)	Safe (0.73)	Safe (0.79)
Acute Oral Toxicity class III	Slightly toxic (0.74)	Slightly toxic (0.60)	Slightly toxic (0.42)
Eye irritation	Irritating (0.97)	Irritating (0.86)	Irritating (0.95)
Genomic Toxicity			
Carcinogenicity	Safe (0.80)	Safe (0.70)	Safe (0.61)
Ames mutagenesis	Caution (0.51)	Safe (0.73)	Safe (0.64)
Eco-Toxicity			
Biodegradation	Safe (0.78)	Safe (0.85)	Safe (0.55)

will aid their metabolism as potential drugs. The human ether a-go-go (hERG) inhibition is related to ventricular arrhythmia, and it can be fatal if a drug is an inhibitor (Sanguinetti and Tristani-firouzi, 2006). The selected compounds are all non-inhibitors of hERG with compound C having the highest probability of not being a hERG inhibitor. However, they all have acute oral toxicity of class three which implies that they are predicted to be slightly toxic and irritating. This irritation is further proposed as they are all eye irritants. Nevertheless, they are all non-carcinogenic with compound A having the highest probability of not being a carcinogen. Though, compound A is also the only one among the selected compounds predicted to be mutagenic. Concerning ecological toxicity, all the selected compounds are biodegradable.

# 3.5. Binding modes and molecular interactions

The binding mode and molecular interaction give insight into the mode of action of the selected ligands to treating SARS-CoV-2. Surprisingly, both the binding mode and the molecular interactions for both VINA and BINDSCOPE are the same for the selected ligands. This is evident in the PDB structures (supporting files attached). The binding mode (Fig. 10) shows the orientation of the ligands in 3-D and the amino residues in the binding pocket. However, the 2-D diagram of the

molecular interactions (Fig. 11) gives more details into the possible mode of action. Compound A has four favorable interactions which include a conventional hydrogen bond with THR 63, a  $\pi$ -alkyl bond with PRO 85, a  $\pi$ -donor hydrogen bond with TRY 269, and a  $\pi$ - $\pi$  Tshaped interaction with PHE 592. Unlike compound A, compound B has one unfavorable interaction with ARG 102 and four favorable interactions. The four favorable interactions for compound B includes one  $\pi$ - $\sigma$ bond with ILE 119, and three  $\pi$ -alkyl bonds with ILE 203, VAL 227 and ILE 1013. Compound C has six favorable interactions; the highest amongst the three selected ligands. These interactions include: three  $\pi$ - $\pi$  T-shaped bonds with ILE 119, ILE 128, and ILE 203; two  $\pi$ -alkyl bonds with VAL 227 and ILE 1013; and one  $\pi$ -sulfur bond with TRP 104. The ranking of the ligands according to BINDSCOPE (Fig. 7), may be based on the number of favorable interactions as compound C which has the highest number of favorable interactions, has the highest topscored, whereas compound B which has one unfavorable interaction is the least scored amongst the three. However, compound A is the only one that has the conventional hydrogen bond interaction which is also the shortest bond length (3.34 Å) of favorable interaction. Hence, it may be the reason it has the highest binding energy according to VINA ranking (Fig. 6) amongst the three selected ligands.

The conservation scores (Table S2) of the amino acid residues



Fig. 10. The 3-D binding modes of the selected ligands ((A) compound A, (B) compound B, and (C) compound C) respectively and their molecular interactions (dashed lines) with the amino residues present in the binding pocket of the spike protein of SARS-CoV-2. The selected ligands are highlighted in yellow.



Fig. 11. The 2-D molecular interactions of the selected ligands ((A) compound A, (B) compound B, and (C) compound C) with the amino residues presnt in the in the binding pocket of the spike protein of SARS-CoV-2. The bond distances are in Angstrom (Å).

involved in the ligand-protein interactions with the selected compounds show that ILE 119, ILE 128, TRP 104 have conservation score of 7 and ILE 1013 has a conservation score of 9. This implies that the amino residues that make specific interactions with the selected compounds are conserved. The PrankWeb tool (Jendele et al., 2019) for binding pocket prediction was also used to validate the amino residue involved in the ligand-protein interaction by comparing the amino residues in the binding pocket of the crystal structure of the spike protein deposited in the protein databank (PDB ID: 6VSB). The amino residues ARG 102 and ARG104 (Fig. 11) which are involved in the ligand-protein interaction are also predicted to be in the binding pockets of 6VSB.

Moreover, to show reliability of the homology model in this study,

Compound A was further docked using AutoDock VINA to a monomer of the experimental crystal structure of the spike protein (PDB ID: 6VSB). The docked ligand of compound A with the experimental structure was then compared to the homology modeled structure (Fig.12). The binding sites are similar as they both occur amidst  $\beta$ pleated sheets and a few  $\alpha$ -helix coil. The 2-D molecular interaction reveals that in the experimental structure, compound A forms a  $\pi$ - $\pi$ interaction with PHE 592 which is similar to what is observed in the homology modeled structure. However, the differences between the experimental and homology modeled structure is the shorter distance of the interaction with PHE 592 which occurs in the former. This is also responsible for the slightly higher docking score of -5.4 kcal/mol



Fig. 12. The binding site of compound A in (A) experimental structure (PDB ID: 6VSB) and (B) homology-modeled structure and 2-D molecular interaction of compound A with the amino residues present in the binding site of (C) experimental structure (PDB ID: 6VSB) and (D) homology-modeled structure The bond distances are in Angstrom (Å).



Fig. 13. The scaffold 1 (A) and scaffold 2 (C) of compound A, and the new structure after lead optimization of scaffold 1 (B) and scaffold 2 (D) respectively. The values below the new structures (B and D) are the drug-likeness values.

observed in the experimental structure. This difference in their molecular interactions is expected as the ligand would have different orientations in both structures. However, the binding of compound A to PHE 592 in both structures validates the homology-model.

# 3.6. Hit-to-Lead optimization

The optimization process often leads to an increase in the binding energy with the target protein, and/or an improvement in the ADMET properties. This process requires structural modifications which improve the functionality of a molecule (Jorgensen, 2009; Maynard et al., 2016; Qiao et al., 2018; Stevens, 2014). The ADMETopt webtool (Yang et al., 2018) was used for the optimization of the three selected compounds based on the ADMET properties to improve the drug-likeness. Compound A has two scaffolds, 1 and 2, as highlighted in Fig. 13. Upon optimization, the best replacement for scaffold one is a pyrol ring with a bromine subsitutent attached to it (Fig. 13B)). This replacement has the highest drug-likeness score of 0.80 (Table S4). For scaffold 2, the furan ring was replaced with a pyrole-like ring attached to a hydroxyl group. This scaffold has the highest drug-like score of 0.76 (Table S5). However, among the two scaffolds, changing scaffold 1 would led to the better optimization because of its higher drug-likeness score. Compound B only has one scaffold which is the di-fluoro benzene ring. Upon undergoing optimization using ADMETopt, the best replacement to give a highest drug-like score of 0.87 (Table S6) is replacing the scaffold with a bromine substituted pyridine (Fig. 14). For compound C, the pyrole ring with a substitued methyl is the scaffold (Fig. 15). However, this was replaced by a thiopene ring having both methyl and bromine



Fig. 14. (a) The scaffold (highlighted) of compound B and (b) its new structure after lead optimization. The values below the new structure denotes the drug-likeness value.



Fig. 15. (a) The scaffold (highlighted) of compound C and (b) its new structure after lead optimization. The values below the new structure denotes the drug-likeness value.

substituents attached to it has the best drug-like score of 0.78 (Table S7). In Hit-to-lead optimization, it is important to note that most times, there is usually a trade-off between improving the binding affinity or improving the drug-likeness of a molecule. In the end, the medicinal chemist needs to compromise on which area to focus on during optimization.

## 4. Conclusions

The recent outbreak of the COVID-19 has put all the health systems in the world on red alert as the virus spreads globally. As there are no known drugs or vaccines to treat this outbreak, developing one is paramount and computer-aided drug design is a useful tool in fasttracking the discovery and development of new drugs that can be used to treat this disease. The consensus scoring approach has been used to combine virtual screening results from both molecular docking and machine learning to select three compounds. These compounds have the potential to inhibit the SARS-CoV-2 glycoprotein which is responsible for virus entry and binding. The molecular docking (VINA) scores of the selected compounds A, B and C are -4.9 kcal/mol, -4.6 kcal/mol and -4.6 kcal/mol, while their corresponding scores from machine learning (BINDSCOPE) are 0.992, 0.989 and 0.993 respectively. Both compounds B and C interact with amino acid residues which are conserved with ILE 1013 which is well conserved in surface glycoprotein. Compound C which has the highest score based on BIN-DSCOPE also has the best oral bioavailability, has all its parameters are within the recommended range. Whereas the ADMET prediction shows that the selected compounds have good absorption and distribution properties and are not carcinogenic. However, their toxicity has to be improved particularly concerning acute oral toxicity and eye irritation. These properties were considered during the Hit-to-Lead optimization which looked at the various scaffolds that can be replaced to improve the drug-likeness and non-toxicity. However, it is important to note that there is usually a trade-off between improving the binding affinity or improving the drug-likeness of a molecule. In the end, the medicinal chemist needs to compromise on which area to focus on during optimization. It is hoped that this work will help other researchers, particularly experimental medicinal scientists in developing a drug that can be used to treat the COVID-19.

# Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

# CRediT authorship contribution statement

Abdulmujeeb T. Onawole: Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Visualization. Kazeem O. Sulaiman: Conceptualization, Data curation, Writing original draft, Writing - review & editing, Visualization, Supervision. Temitope U. Kolapo: Methodology, Writing - original draft, Writing review & editing, Visualization. Fatimo O. Akinde: Methodology, Writing - original draft. Rukayat O. Adegoke: Conceptualization, Methodology, Writing - original draft, Writing - review & editing.

## **Declaration of Competing Interest**

The authors declare no conflict of interest.

# Acknowledgments

The authors would like to acknowledge all the scientists, researchers, and health workers working on finding an immediate solution to treat the COVID-19, most especially those that worked to make the full genome of the virus available in a short time. Their effort is quite astonishing. They have provided the bedrock for other researchers to build on.

# Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.virusres.2020.198022.

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